Acidic colonic microclimate – possible reason for false negative hydrogen breath tests

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SUMMARY About 5% of normal subjects fail to produce increased hydrogen breath concentration after ingestion of the non-digestible carbohydrate lactulose (low hydrogen producers). The existence of low hydrogen producers limits the diagnostic use of hydrogen (H_2) breath tests. We studied the effects of lactulose and of magnesium sulphate ($MgSO_4$) pretreatment on stool-pH and on hydrogen exhalation after oral loading with lactulose or lactose in 17 hydrogen producers and 12 low hydrogen producers. In seven hydrogen producers acidification of stool pH by lactulose pretreatment (20 g tid) decreased hydrogen exhalation and three of seven (43%) became low hydrogen producers. In contrast, after pretreatment of eight low hydrogen producers with magnesium sulphate (5 g twice daily) all eight produced hydrogen after a lactulose load. Similarly four lactose intolerant low hydrogen producers had abnormal lactose hydrogen breath tests after $MgSO_4$ pretreatment. $MgSO_4$ pretreatment neither resulted in false positive lactose hydrogen breath tests in five lactose tolerant hydrogen producers, nor increased the hydrogen exhalation in five additional hydrogen producing controls after ingestion of lactulose. The results of these studies confirm that hydrogen production from lactulose decreases when the colonic pH is lower (lactulose pretreatment), and increases when colonic pH is higher ($MgSO_4$ pretreatment). In low hydrogen producers the lacking increase of H_2 exhalation after ingestion of non-digestible carbohydrates can be overcome by $MgSO_4$ pretreatment, thus increasing the sensitivity of the test by avoiding false negative hydrogen breath tests in low hydrogen producers with disaccharide malabsorption or maldigestion. The underlying mechanism of this remarkable effect of $MgSO_4$ pretreatment warrants further investigation.

The hydrogen breath test is a valuable non-invasive diagnostic tool to detect carbohydrate malabsorption and bacterial overgrowth in the small bowel and to determine the gastrointestinal transit time. Metabolism of non-absorbed carbohydrates by gut bacteria results in the production of short chain fatty acids, CO_2 and H_2 . About 20% of the formed H_2 is absorbed from the gut and exhaled by the lungs. The H_2 concentration in breath can be measured by gas chromatography or electrochemically.¹ The magnitude of the H_2 excretion in the breath is influenced by a variety of factors such as smoking,² diet,³ and antibiotic treatment.⁴ The number of colonic bacteria and the composition of colonic flora may change the H_2 excretion as well as the metabolic pathways mainly used by the colonic flora for degradation of carbohydrates.^{5,6} About 5% of normal subjects fail to

show an increase of more than 20 ppm in H_2 excretion in breath after the ingestion of lactulose.^{7,8} Therefore, the H_2 breath test cannot be generally accepted as a noninvasive screening test for carbohydrate malabsorption. The reason for the lack of H_2 production by gut bacteria in these patients is unclear. The H_2 production from glucose by faecal incubates is pH dependent *in vitro*, the pH-optimum being at pH 7.0–7.45.⁹ *In vivo*, oral administration of lactulose – a non-digestible carbohydrate – produces a marked acidification of proximal colonic contents¹⁰ and results in a decrease of H_2 excretion in breath after oral lactulose loading.⁹ It was concluded, therefore, that the bacterial metabolism of lactulose and of other malabsorbed or maldigested carbohydrates and the H_2 production may be inhibited at low pH. In lactose intolerant patients or in patients treated with non-digestible disaccharides, repeated ingestion of such carbohydrates may lead to a low colonic pH because of bacterial degradation of carbohydrates to

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organic acids in the colon. We therefore tested the possibility of overcoming the low colonic pH by oral MgSO_4 application and investigated the effect of oral MgSO_4 pretreatment on faecal pH and on hydrogen breath tests in general and particularly in low hydrogen producers.

Methods

PATIENTS

The H_2 production status was determined routinely by lactulose H_2 breath tests in patients with various gastrointestinal or hepatic diseases. After exclusion of patients with chronic inflammatory bowel diseases and with previous surgery of the gastrointestinal tract 17 hydrogen producers (seven with stable cirrhosis of the liver, five with duodenal ulcer, five healthy controls), and 12 low hydrogen producers (eight patients with stable cirrhosis of the liver, two with coeliac disease, two with isolated lactase deficiency) consented to participate in additional H_2 breath tests (study protocol, Table 1). Of these 29 patients 22 were men and seven women. They had not been on antibiotics during the last four weeks before and during the study. In seven hydrogen producers the lactulose H_2 breath test was repeated after pretreatment with lactulose (20 g tid for three days) and in five hydrogen producers after pretreatment with MgSO_4 (5 g dissolved in 200 ml tap water, given orally twice on the day before the second test). In eight low hydrogen producers the lactulose H_2 breath test was repeated after MgSO_4 pretreatment. In addition, lactose H_2 breath tests were carried out in five lactose tolerant hydrogen producers and in four lactose intolerant low hydrogen producers and the tests were repeated after MgSO_4 pretreatment. A fresh stool specimen was obtained for pH determination from each patient in the morning before each H_2 breath test and was worked up immediately.

LACTULOSE/LACTOSE H_2 BREATH TEST

After an overnight fast the patients received 20 g lactulose (Laevolac-Lactulose-Konzentrat, Fa

Laevosan, Linz, Austria) dissolved in 250 ml herbal tea or 50 g of lactose in 500 ml herbal tea. Before and after the ingestion of the disaccharide the endexpiratory air was collected with the 'single breath technique'¹¹ in 15 minutes intervals for four hours. H_2 concentration in the breath was determined by an electrochemical cell (Stimotron, Wendelstein, FRG). Its accuracy is $\pm 2\%$, the sensitivity is 2 ppm and it gives a linear response to breath hydrogen between 0 and 250 ppm. In addition, blood glucose was measured before and in 15 minutes intervals after lactose ingestion. A blood glucose rise of less than 20 mg% above the baseline was judged diagnostic for lactose malabsorption.¹²

MEASUREMENT OF THE FAECAL pH

The faecal pH was determined in a smear of fresh stool by two indicator stripes (Merck, Darmstadt, FRG) as described.¹³ The stripe used first had an accuracy of measurement of 1 in a range of pH 0–14, the second one had an accuracy of 0.2 in the range of pH 4.0–8.0.

STATISTICAL ANALYSIS

The statistical calculations and the graphs were done by SAS (Statistical Analysis System of SAS Inst Inc, Cary, North Carolina) on the computer. The comparison of the means of both groups and tests was calculated by an unpaired twotailed Student's *t* test. The maximal rise of H_2 excretion (max-H_2) in ppm (parts per million) above basal value and the area under the curve (AUC-H_2) in $\text{ppm} \times \text{min}$ – approximated by the rectangle rule – were calculated on the computer. AUC-H_2 is the area between the exhalation curve and the extrapolated basal value. If the maximal H_2 increase was less than 20 ppm after lactulose ingestion, the subject was defined as a low hydrogen producer.

Results

EFFECTS OF pH MODULATION OF COLONIC CONTENTS ON LACTULOSE H_2 BREATH TESTS IN HYDROGEN PRODUCERS

A three day treatment with 20 g lactulose (tid) resulted in a markedly decreased production of H_2 after ingestion of 20 g lactulose (Fig. 1) with lower max-H_2 and smaller AUC-H_2 (Table 2). Three patients (43%) became low hydrogen producers by lactulose pretreatment. The stool pH was lower after lactulose pretreatment (5.1 ± 0.2 v 5.8 ± 0.2 ; $p < 0.05$). MgSO_4 pretreatment had no effect on H_2 production after ingestion of lactulose (Table 2) and did not change the stool pH (6.4 ± 0.4 v 6.6 ± 0.3). The oro-caecal transit time did not decrease significantly (111 ± 44 v 128 ± 39 min; $p = 0.48$).

Table 1 Study protocol

29 Patients	
17 H_2 -producers	12 low H_2 -producers
Lactulose H_2 breath test after 7	Lactulose H_2 breath test after 8
lactulose pretreatment	MgSO_4 pretreatment
Lactulose H_2 breath test after 5	Lactose H_2 breath test before 4
MgSO_4 pretreatment	and after MgSO_4
Lactose H_2 breath test before 5	pretreatment
and after MgSO_4	
pretreatment

Fig. 1 Lactulose H_2 breath test (mean $\pm$ SEM) before ($\square$ — $\square$) and after ($\bullet$ — $\bullet$) treatment with 20 g lactulose (tid) in seven hydrogen producers.

EFFECTS OF $MgSO_4$ TREATMENT ON LACTULOSE H_2 BREATH TESTS IN LOW HYDROGEN PRODUCERS

The $MgSO_4$ pretreatment led to a steeper H_2 exhalation curve (Fig. 2) with higher max- H_2 and larger AUC- H_2 (Table 2) and to an increased stool pH (7.2 ± 0.3 v 6.1 ± 0.3 ; $p < 0.05$). All eight low hydrogen producers became hydrogen producers after $MgSO_4$ pretreatment.

EFFECTS OF $MgSO_4$ TREATMENT ON LACTULOSE H_2 BREATH TEST IN LACTULOSE TOLERANT HYDROGEN PRODUCERS AND IN LACTULOSE INTOLERANT LOW HYDROGEN PRODUCERS

The $MgSO_4$ pretreatment had no effect on the max- H_2 or AUC- H_2 in lactulose tolerant hydrogen pro-

ducers after lactulose ingestion (Table 3, Fig. 3) or on stool pH (6.0 ± 0.1 v 6.2 ± 0.4). In contrast, $MgSO_4$ pretreatment resulted in increased max- H_2 and AUC- H_2 after lactulose ingestion in lactulose intolerant low hydrogen producers (Table 3, Fig. 4), thus all four patients became hydrogen producers and the lactulose H_2 breath test was diagnostic for lactulose malabsorption. The stool pH increased (5.9 ± 0.2 v 5.2 ± 0.1 ; $p < 0.05$) after $MgSO_4$ pretreatment.

Discussion

The causes for a low hydrogen producer state are not clear at present. Several factors such as the amount of given disaccharides, the functional status of the small bowel, and the colonic microclimate may affect the

Table 2 Lactulose H_2 breath test before (baseline) and after lactulose or $MgSO_4$ pretreatment

	n	Baseline		After lactulose		After $MgSO_4$	
		Max- H_2	AUC- H_2	Max- H_2	AUC- H_2	Max- H_2	AUC- H_2
H_2 producers	7	69 ± 12	8523 ± 1646	$26 \pm 10^*$	$5414 \pm 1904^*$	60 ± 16	9744 ± 2002
H_2 producers	5	51 ± 7	7385 ± 917			$52 \pm 19^*$	$5420 \pm 852^*$
Low H_2 producers	8	12 ± 2	2347 ± 369				

Every parameter as $\bar{x} \pm SEM$; max- H_2 in ppm; AUC- H_2 in ppm $\times$ min; $^*p < 0.05$, *t* test, in comparison with the corresponding baseline.

Fig. 2 Lactulose H_2 breath test in eight low-hydrogen producers without ($\square-\square$; mean + SEM) and in the same patients with ($\bullet-\bullet$; mean - SEM) pretreatment with $MgSO_4$ (2×5 g).

magnitude of bacterial H_2 production in the colon. The incidence of low hydrogen producers in the normal population varies considerably from 0% to 20%.^{5,7,8,13} Regional or ethnical differences are mostly the result of different dietary habits and this may explain the different frequencies of low hydrogen producers in several studies. In addition, they can be attributed to different criteria applied to define low or non-hydrogen producers or flat H_2 breath tests by various authors. In this study low hydrogen producers were defined by a lack of an increase of H_2

concentration in the breath of greater than 20 ppm after an ingestion of 20 g lactulose. Using our rather strict criteria the incidence of low hydrogen producers in the Austrian population is less than 5%.

As the H_2 production from disaccharides is a result of bacterial metabolism in the colon, the magnitude of H_2 production presumably is sensitive to changes in the composition of the colonic microflora and in the colonic microclimate which are interrelated. Faecal hydrogen production does not seem to be a good indicator for *in vivo* hydrogen producing

Fig. 3 Lactose H_2 breath test (mean) without ($\square-\square$) and with ($\bullet-\bullet$) $MgSO_4$ pretreatment (2×5 g) in five hydrogen producers.

Table 3 Lactose H₂ breath test before (baseline) and after MgSO₄ pretreatment

	n	Baseline		After MgSO ₄	
		Max-H ₂	AUC-H ₂	Max-H ₂	AUC-H ₂
Lactose tolerant H ₂ producers	5	6±1	894±473	4±2	880±336
Lactose intolerant low H ₂ producers	4	6±2	1532±834	86±27*	15 553±3064*

Every parameter as $\bar{x} \pm \text{SEM}$; max-H₂ in ppm; AUC-H₂ in ppm×min; *p<0.05, in comparison with the corresponding baseline values.

capability,¹⁴ but it was shown that the faecal H₂ production is pH dependent.⁹ In carbohydrate malabsorption or maldigestion or in subjects treated with non-digestible disaccharides the colonic microclimate will become acidic.⁹ Chronic ingestion of non-digestible disaccharides results in stimulation of acid producing bacterial enzymes such as β -galactosidases⁶ and caecal pH will decrease faster after disaccharide ingestion. At low pH the growth and metabolism of many microorganisms are inhibited⁵ and the portion of bacteria consuming or not producing H₂ such as *Lactobacillus*, *Bifidobacterium* and *Eubacteria* will increase^{5,6} which prefer acidic milieu.¹⁵ Thus, patients with carbohydrate malabsorption or maldigestion who consume a diet rich of non-digestible/non-absorbable carbo-

hydrates could frequently be low hydrogen producers; this matter considerably limits the diagnostic value of H₂ breath tests in this interesting group of patients.

The results of this study show that the failure to produce H₂ from malabsorbed or non-digestible carbohydrates can be overcome by pretreatment with MgSO₄. Thereby, the low hydrogen producer state can be eliminated and the diagnostic value of the H₂ breath test for detecting carbohydrate malabsorption or for measuring oro-caecal transit time can be improved. The mechanism by which MgSO₄ pretreatment enhances or facilitates intestinal H₂ production and H₂ exhalation in low hydrogen producers, is not clear. Possible ways of action could involve effects on intestinal motility and transit time

Fig. 4 Lactose H₂ breath test without (□—□; mean+SEM) and with (●—●; mean-SEM) MgSO₄ pretreatment (2×5 g) in four low hydrogen producers.

as well as on intestinal bicarbonate secretion resulting in alterations of the colonic microclimate in favour of bacterial H_2 production, absorption and exhalation. The pH increasing effect of $MgSO_4$ could be reached by its cathartic effect cleansing the caecum from carbohydrates which are the substrates for acid producing bacteria, without itself being substrate for bacterial metabolism in contrast to lactulose. Unfortunately our data do not permit a conclusive explanation of the enhancing effect of $MgSO_4$ pretreatment on H_2 exhalation which we observed particularly striking in lactose intolerant low hydrogen producers. As the stool pH increased after $MgSO_4$ pretreatment from pH 6.1 to pH 7.2 a pH-mediated mechanism appears likely to be operative. Additional studies including colonic pH monitoring are, however, needed to clarify definitely a possible pH mediated effect, as stool pH does not reflect the pH in different parts of the colon.¹⁰ In addition, stool pH also depends on the caecal anal transit time. The colonic pH increases from the caecum to the rectum¹⁰ and thus a shorter caecal anal transit time will be associated with lower stool pH. Therefore, in hydrogen producers without diarrhoea at the beginning of the study the stool pH is hardly comparable to that after treatment with cathartics such as $MgSO_4$ or lactulose. The pH in the colon ascendens can be accurately measured by radiotelemetry using pH detectors in capsules or by plastic tubes swallowed by the patient and their location controlled by radiography. Using this technique it was shown that lactulose treatment decreases pH in colon ascendens below 5.¹⁰ These techniques, however, cannot be used routinely in patients screened for carbohydrate malabsorption. Treatment with $MgSO_4$ was shown by radiotelemetry to increase colonic pH.¹⁰ Thereby, the conditions for bacterial H_2 production can be optimised. In addition, there was a tendency to decrease the oro-caecal transit time by $MgSO_4$ pretreatment, thus more substrates for bacterial H_2 production could reach the colon¹⁶ and the magnitude of hydrogen production could be increased by a higher rate of entry of unabsorbed carbohydrates into the caecum;¹⁷ but this may be only a side effect of $MgSO_4$ pretreatment, the main effect probably being a pH mediated change of the metabolism of colonic bacteria.

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